CLAIMS

1. A method of amplifying RNA sequences complementary to one or more than one target polynucleotide that is single stranded or made single stranded, comprising

a) forming double stranded cDNA templates containing sequences present in said target polynucleotide, wherein said sequences are operably linked to a promoter region, by

i) annealing said single stranded target polynucleotide with a first oligonucleotide comprising a primer operably linked to a promoter region to form a first complex,

ii) synthesizing a first strand cDNA by reverse transcription of said first complex,

iii) degrading first oligonucleotides not used in i) or ii) above with exonuclease activity,

iv) annealing said first strand cDNA, after denaturing the mRNA/cDNA hybrid or degrading the RNA from said hybrid, with a plurality of second oligonucleotides comprising a random primer region to form a population of second complexes, and

v) forming double stranded cDNA templates from said population of second complexes with DNA polymerase activity; and

b) transcribing said cDNA templates with an RNA polymerase capable of initiating transcription via said promoter region to produce amplified RNA (aRNA) containing sequences complementary to said target polynucleotide.

2. The method of claim 1 wherein said target polynucleotide is mRNA.

3 The method of claim 1 wherein said more than one target polynucleotide are a cellular mRNA preparation.

4. The method of claim 1 wherein said first oligonucleotide comprises a primer containing an oligo or poly dT sequence.

5. The method of claim 4 wherein said oligo or poly dT sequence is at least about eight dT in length.

6. The method of claim 1 wherein said random primer region comprises at least about six random nucleotides.

7. The method of claim 6 wherein said random primer region comprises at least about nine random nucleotides.

8. The method of claim 1 wherein said DNA polymerase activity is DNA dependent.

9. The method of claim 8 wherein said DNA dependent polymerase activity is selected from exonuclease deficient Klenow, Taq polymerase activities, and combinations thereof.

10. The method of any of claims 1-8 wherein the amplification of RNA sequences complementary to one or more than one target polynucleotide is increased by preparing additional double stranded DNA templates, comprising all or part of the sequence of the aRNA, and initiating transcription from the additional templates, said method comprising

annealing said aRNA to a third oligonucleotide comprising a primer region to form a third complex,

synthesizing the first strand of said additional double stranded DNA templates by reverse transcription of said third complex,

annealing said first strand of additional DNA templates, after denaturing the aRNA/DNA hybrids or degrading the aRNA from said hybrids,

with said first oligonucleotide comprising an operably linked promoter region to form a fourth complex,

forming additional double stranded DNA templates from said fourth complex with DNA dependent DNA polymerase activity, and

transcribing said double stranded DNA templates with an RNA polymerase capable of initiating transcription via said promoter region to produce additional amplified RNA (aRNA) containing sequences complementary to said target polynucleotide,

wherein the above annealing, synthesizing, annealing, forming and/or transcribing components of the method are optionally repeated to further amplify said RNA sequences complementary to one or more than one target polynucleotide.

11. The method of claim 10 wherein said third oligonucleotide comprises a random primer region.

12. The method of claim 11 wherein said random primer region comprises at least about six random nucleotides.

13. The method of claim 12 wherein said random primer region comprises at least about nine random nucleotides.

14. The method of claim 10 wherein said DNA dependent DNA polymerase activity comprises exonuclease deficient Klenow and Taq polymerase activities.

15. The method of claim 10 wherein said third oligonucleotide comprises a known primer sequence.

16. The method of claim 15 wherein said known primer sequence is complementary to the 3' region of said aRNA.

17. A method of amplifying RNA sequences complementary to, or present in, one or more than one target polynucleotide that is single stranded or made single stranded, comprising

a) forming double stranded cDNA templates containing sequences present in said target polynucleotide, wherein said sequences are operably linked to a promoter region, by

i) annealing said single stranded target polynucleotide with a first oligonucleotide comprising a primer operably linked to a promoter region to form a first complex,

ii) synthesizing a first strand cDNA by reverse transcription of said first complex,

iii) degrading first oligonucleotides not used in i) or ii) above with exonuclease activity,

iv) annealing said first stranded cDNA, after denaturing the mRNA/cDNA hybrid or degrading the RNA from said hybrid, with a plurality of second oligonucleotides comprising a random primer region to form a population of second complexes, and

v) forming double stranded cDNA templates from said population of second complexes with DNA dependent DNA polymerase activity; and

b) transcribing said cDNA templates with an RNA polymerase capable of initiating transcription via said promoter region to produce amplified RNA (aRNA) containing sequences complementary to said target polynucleotide;

c) forming additional double stranded DNA templates from said aRNA by

i) annealing said aRNA with a third oligonucleotide comprising a primer region operably linked to a promoter region to form a third complex,

ii) synthesizing the first strand of said additional DNA template by reverse transcription of said third complex,

iii) annealing said first strand of additional DNA template, after denaturing the aRNA/DNA hybrid or degrading the aRNA from said hybrid, with said first oligonucleotide to form a population of fourth complexes, and

iv) forming additional double stranded DNA templates from said population of fourth complexes with DNA dependent DNA polymerase activity; and

d) transcribing said additional DNA templates with an RNA polymerase capable of initiating transcription via the promoter region of said first oligonucleotide to produce amplified RNA (aRNA) containing sequences complementary to said target polynucleotide or via the promoter region of said third oligonucleotide to produce aRNA containing sequences present in said target polynucleotide.

18. The method of claim 17 wherein said formation of additional double stranded DNA templates from said aRNA further comprises degrading third oligonucleotides not used in c) i) or c) ii) with exonuclease activity before forming additional double stranded DNA templates.

19. The method of claim 17 wherein said target polynucleotide is mRNA.

20. The method of claim 17 wherein said more than one target polynucleotide are a cellular mRNA preparation.

21. The method of claim 17 wherein said first oligonucleotide comprises a primer containing an oligo or poly dT sequence.

22. The method of claim 21 wherein said oligo or poly dT sequence is at least about eight dT in length.

23. The method of claim 17 wherein said random primer region comprises at least about six random nucleotides.

24. The method of claim 23 wherein said random primer region comprises at least about nine random nucleotides.

25. The method of claim 17 wherein said DNA dependent DNA polymerase activity comprises exonuclease deficient Klenow and Taq polymerase activities.

26. The method of claim 17 wherein said third oligonucleotide comprises a random primer region.

27. The method of claim 26 wherein said random primer region comprises at least about six random nucleotides.

28. The method of claim 27 wherein said random primer region comprises at least about nine random nucleotides.

29. The method of claim 17 wherein said third oligonucleotide comprises a known primer sequence.

30. The method of claim 29 wherein said known primer sequence is complementary to the 3' region of said aRNA.

31. The method of claim 1, 10 or 17 wherein said first oligonucleotide comprises a T7 promoter region.

32. The method of claim 17 wherein said third oligonucleotide comprises a T3 or SP6 promoter region.